Information *and* Technology Sharing

A Cooperative Endeavor between *USEPA Region 3*

-and-

USEPA Office of Research and Development
National Exposure Research Laboratory
Ecological Exposure Research Division

David Lattier

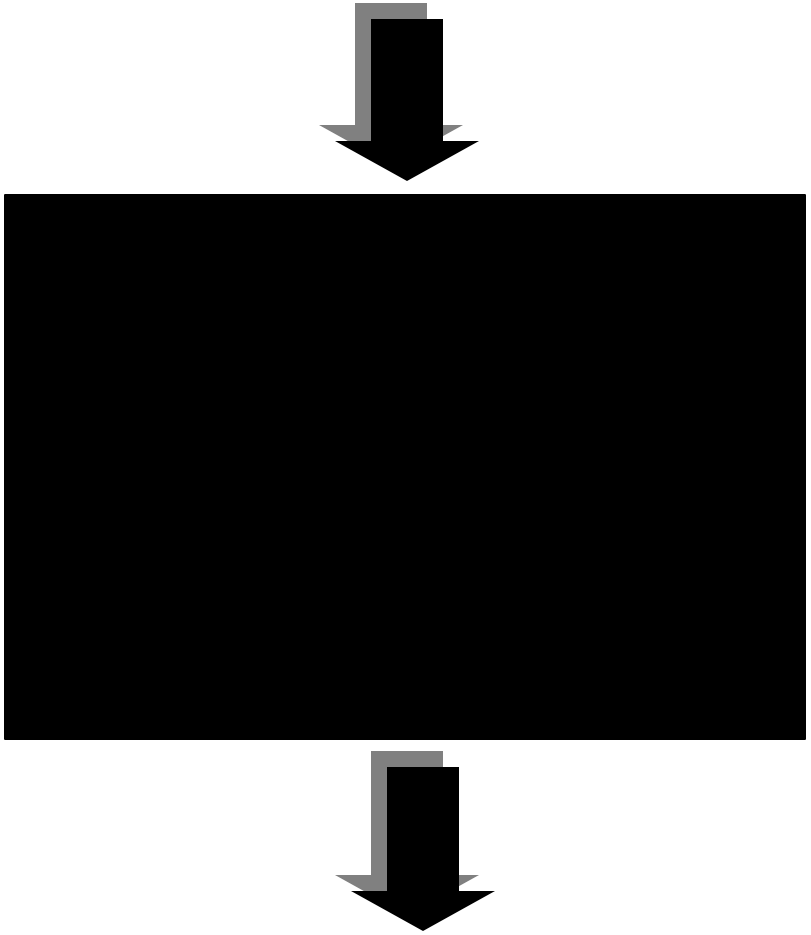
Who and *What*…

Molecular Ecology Research Branch

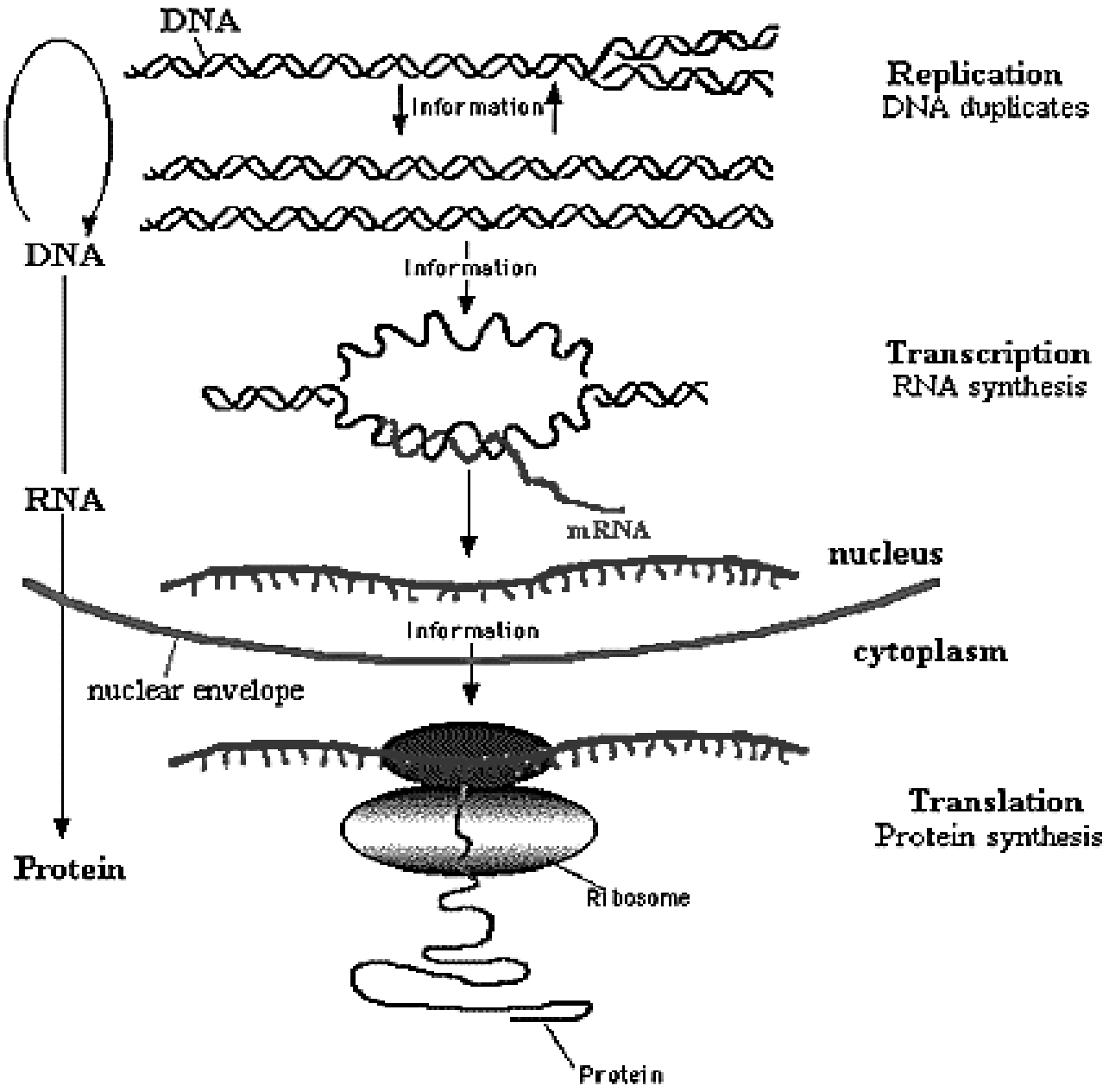
Cincinnati, Ohio

The Perfect Marriage…

GENE



PROTEIN



The Central Dogma of Molecular Biology

The *Central Dogma*

- Central dogma of molecular biology states that DNA carries the genetic information which is transcribed to RNA and subsequently translated to protein

Can we make sense of the complexities, and the seeming ambiguity of cellular processes?

Humans…

10^{13} Cells! - each of which contains the identical complement of DNA… approximately 3.2 x 10^{9} nucleotide pairs!

…identical DNA, but diverse cell types and tissue functions! Huh!?

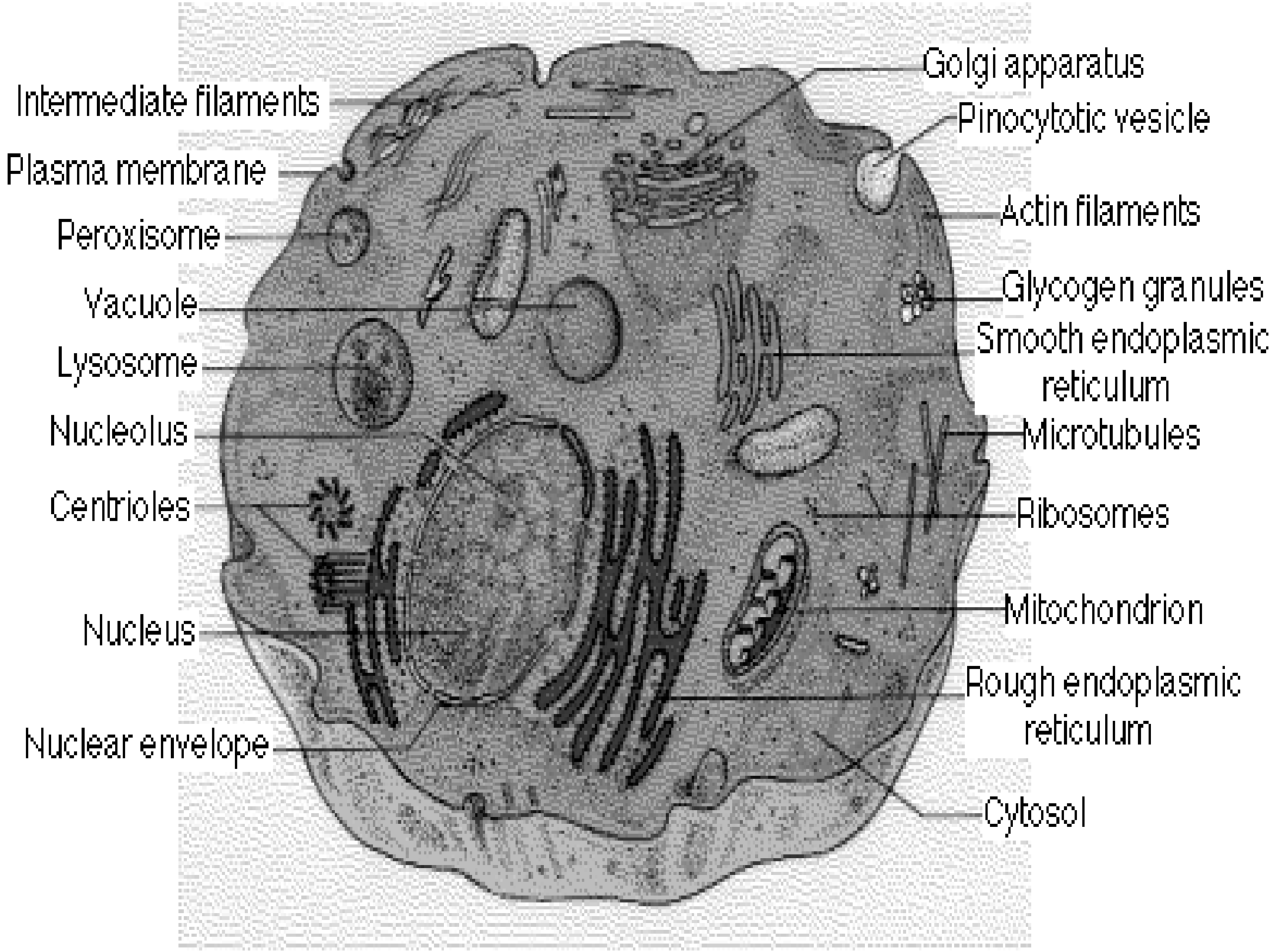
…of 3.2 x 10^{9} nucleotide pairs, *only* 2% used in the production of proteins!

The *Genome*

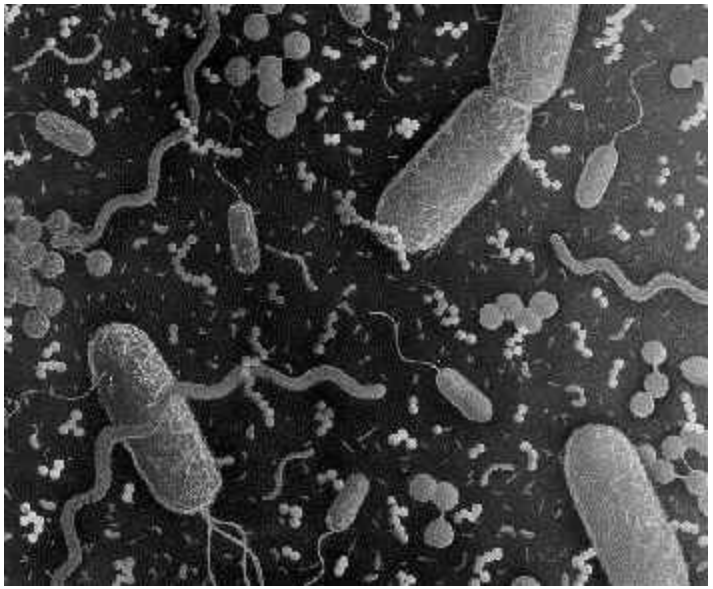
- The genome is the total genetic content of an organism
- Genome size varies widely among organisms
  - Does not correlate with complexity
  - Tulips have **10x** amount of DNA as humans
  - One species of amoeba has **100x** as much DNA as humans
  - Pufferfish genome **1/8** the size of humans
- Genome is full of extra DNA

Consider this…

~ 100 million species on the planet, and even though they share the same biochemical '*letters*' in their cellular alphabet [nucleotides and amino acids], the *order* of those letters varies, thereby forming different words…*leading to vastly unique, yet fascinating stories*…


Intermediate filaments
Plasma membrane
Peroxisome
Vacuole
Lysosome
Nucleolus
Centrioles
Nucleus
Nuclear envelope
Golgi apparatus
Pinocytotic vesicle
Actin filaments
Glycogen granules
Smooth endoplasmic reticulum
Microtubules
Ribosomes
Mitochondrion
Rough endoplasmic reticulum
Cytosol

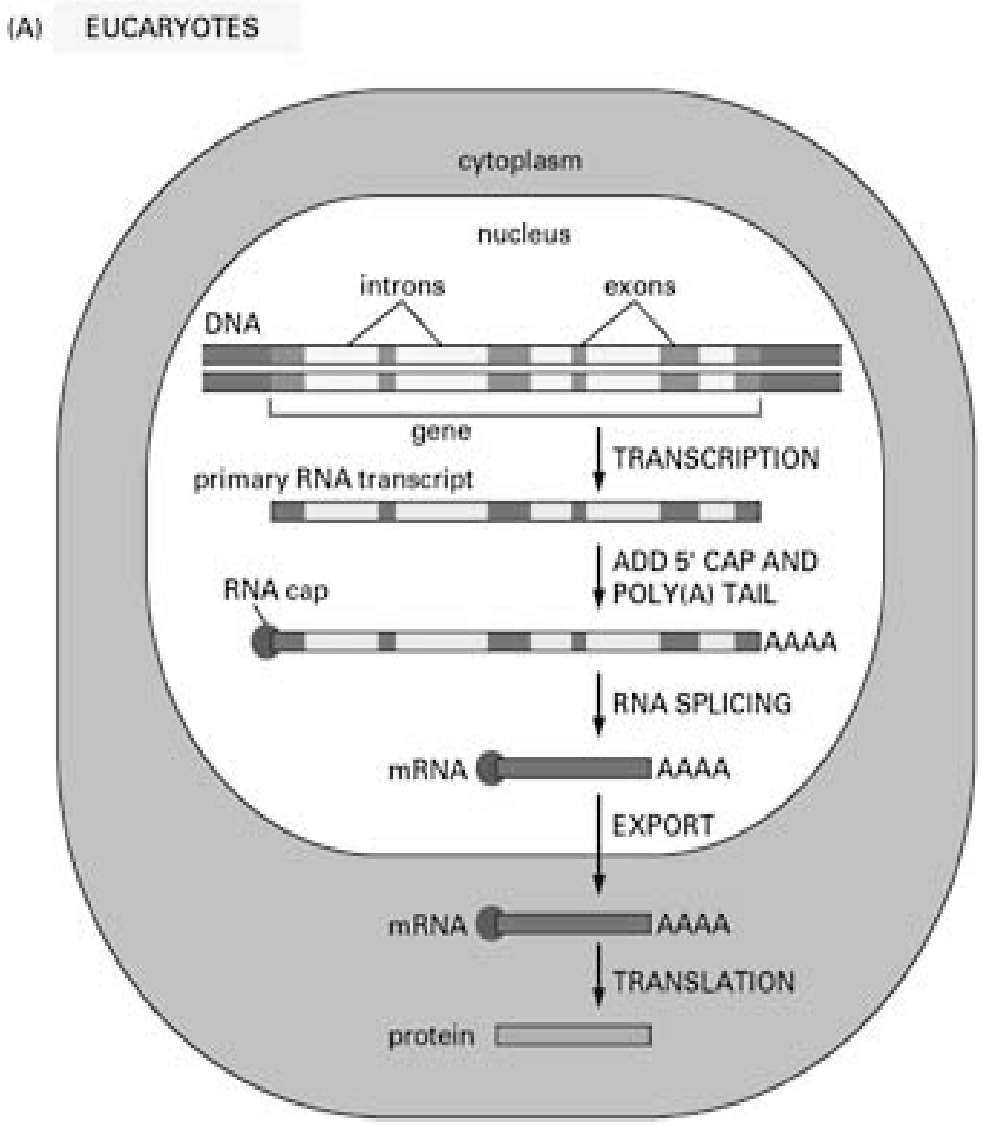
Eukaryotes -*vs*- Prokaryotes

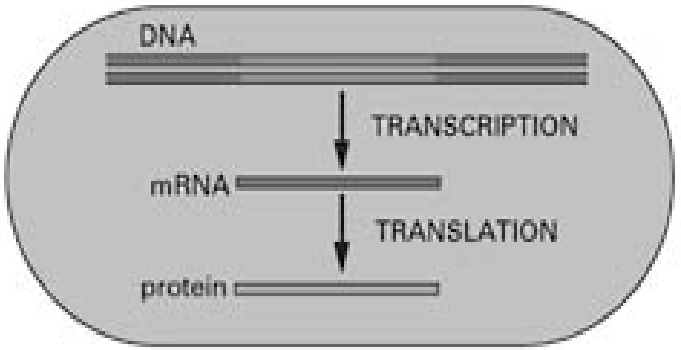


Bugs have no…

- Cellular compartmentalization
- Nucleus
- …business on the planet!

Eukaryotes -*vs*- Prokaryotes





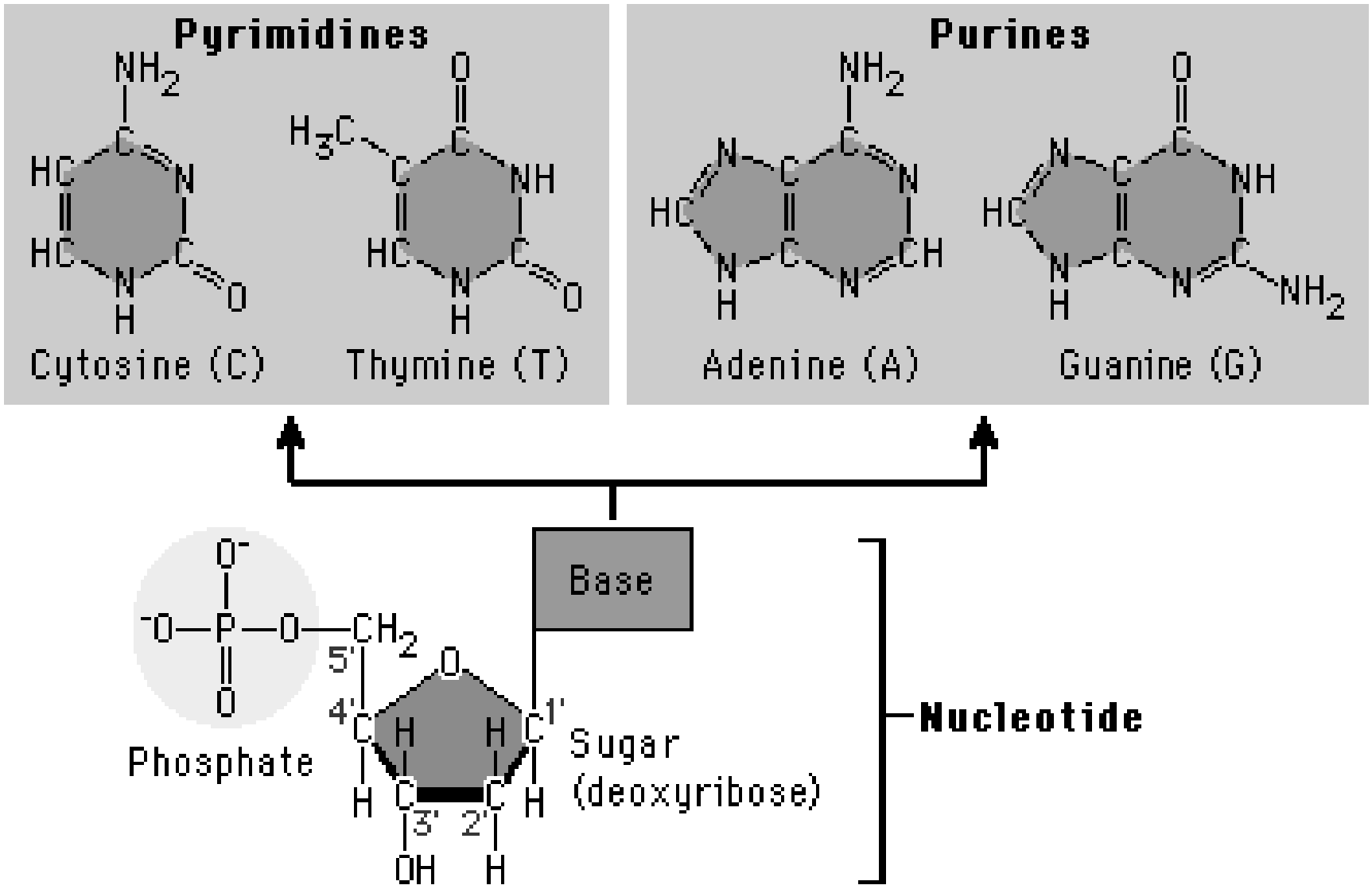
Nucleic Acids

- Originally isolated from nuclei
- Two types
  - **Deoxyribonucleic acid** (DNA)
    - Passed from generation to generation
    - Constitute genome/chromosomes/genes
    - Contains instructions for making proteins
  - **Ribonucleic acid** (RNA)
    - Function in the synthesis of proteins coded by DNA
    - Several distinct types
    - Make up the genomes of some viruses

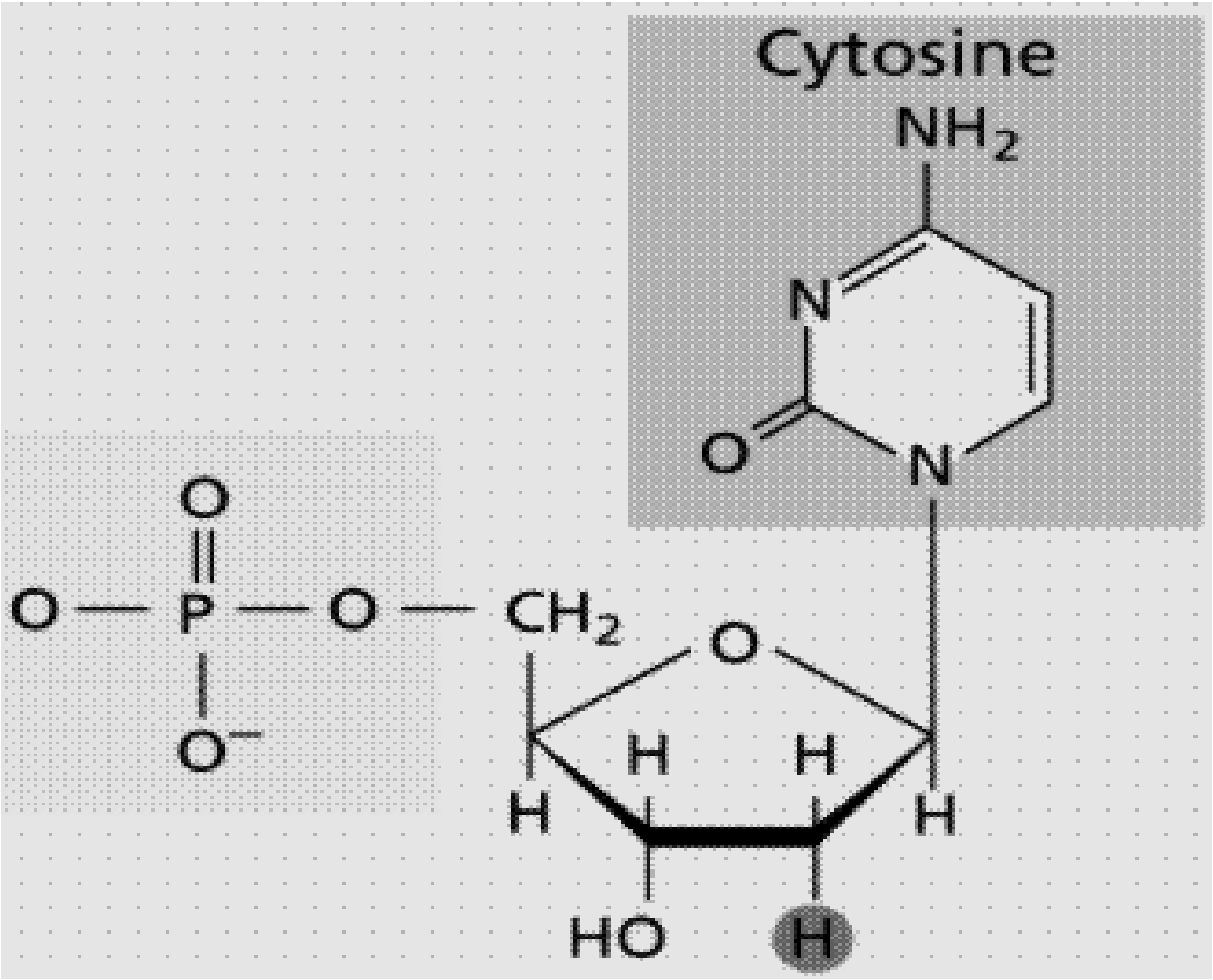
Overview of *DNA* and *RNA*

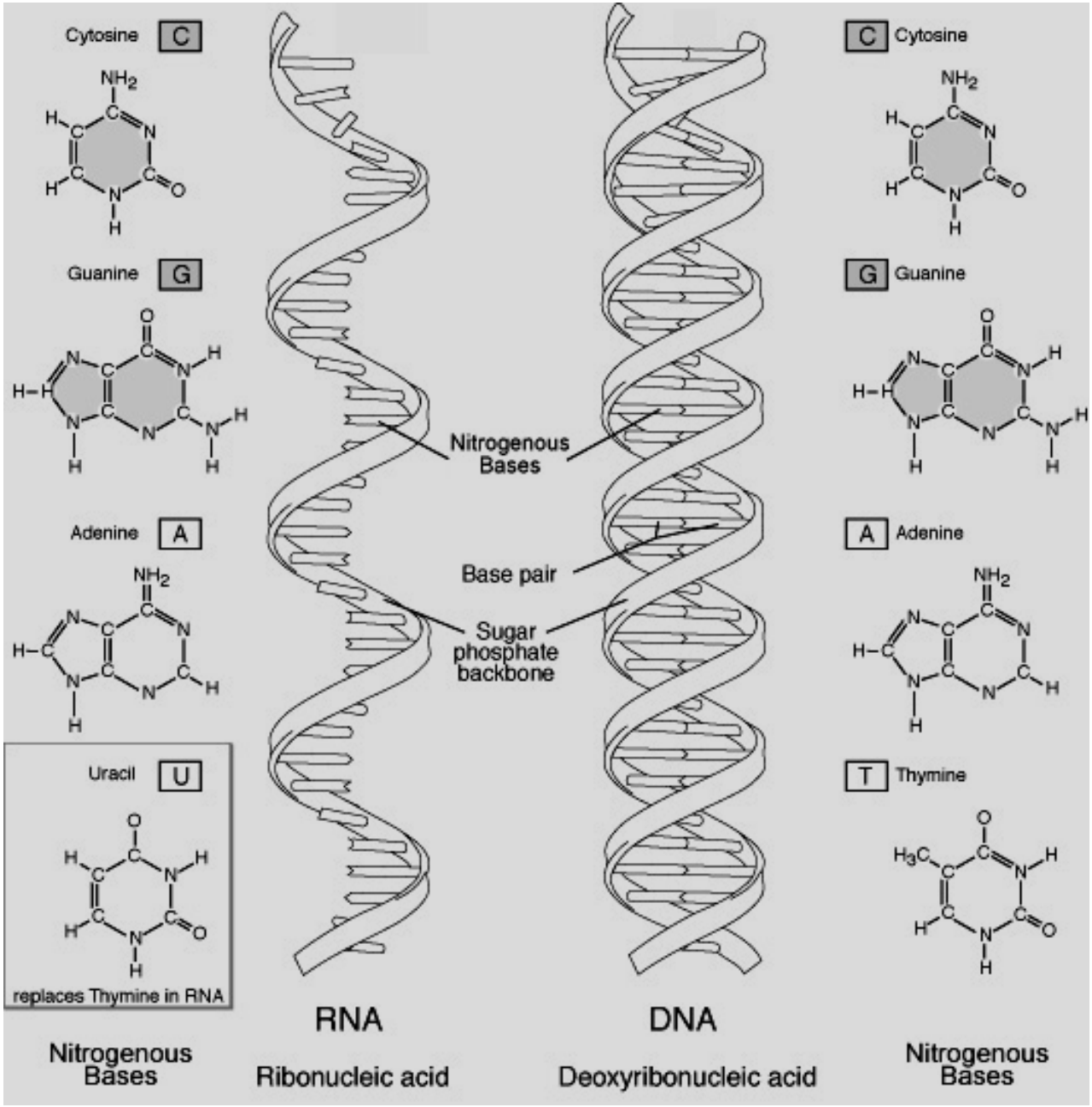
- DNA is a double helix
- DNA and RNA are chains of nucleotides
- DNA stores information
- DNA and RNA are similar (but different)
- The two strands of the DNA helix are aligned in opposite directions

Nucleotide structure

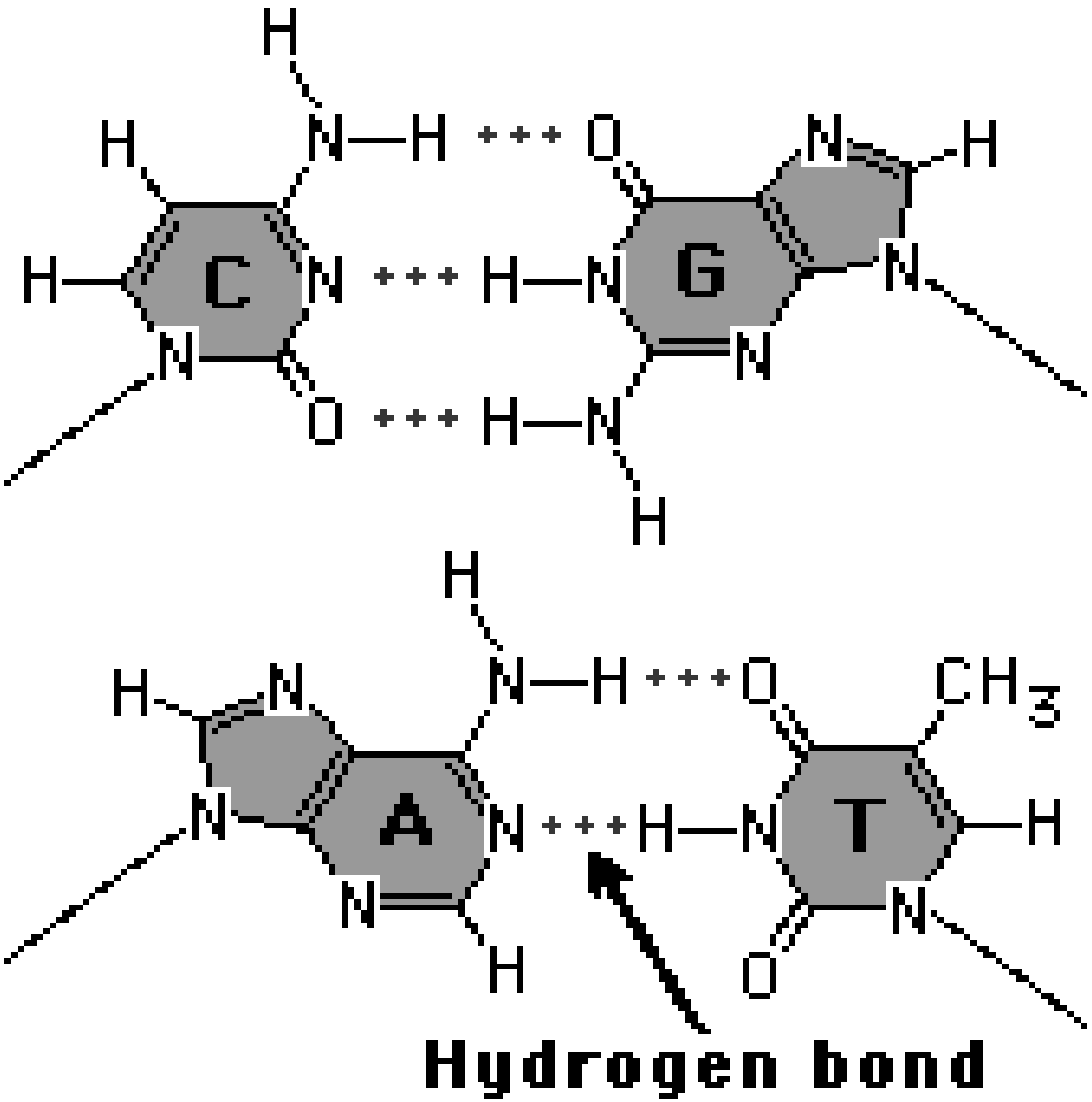


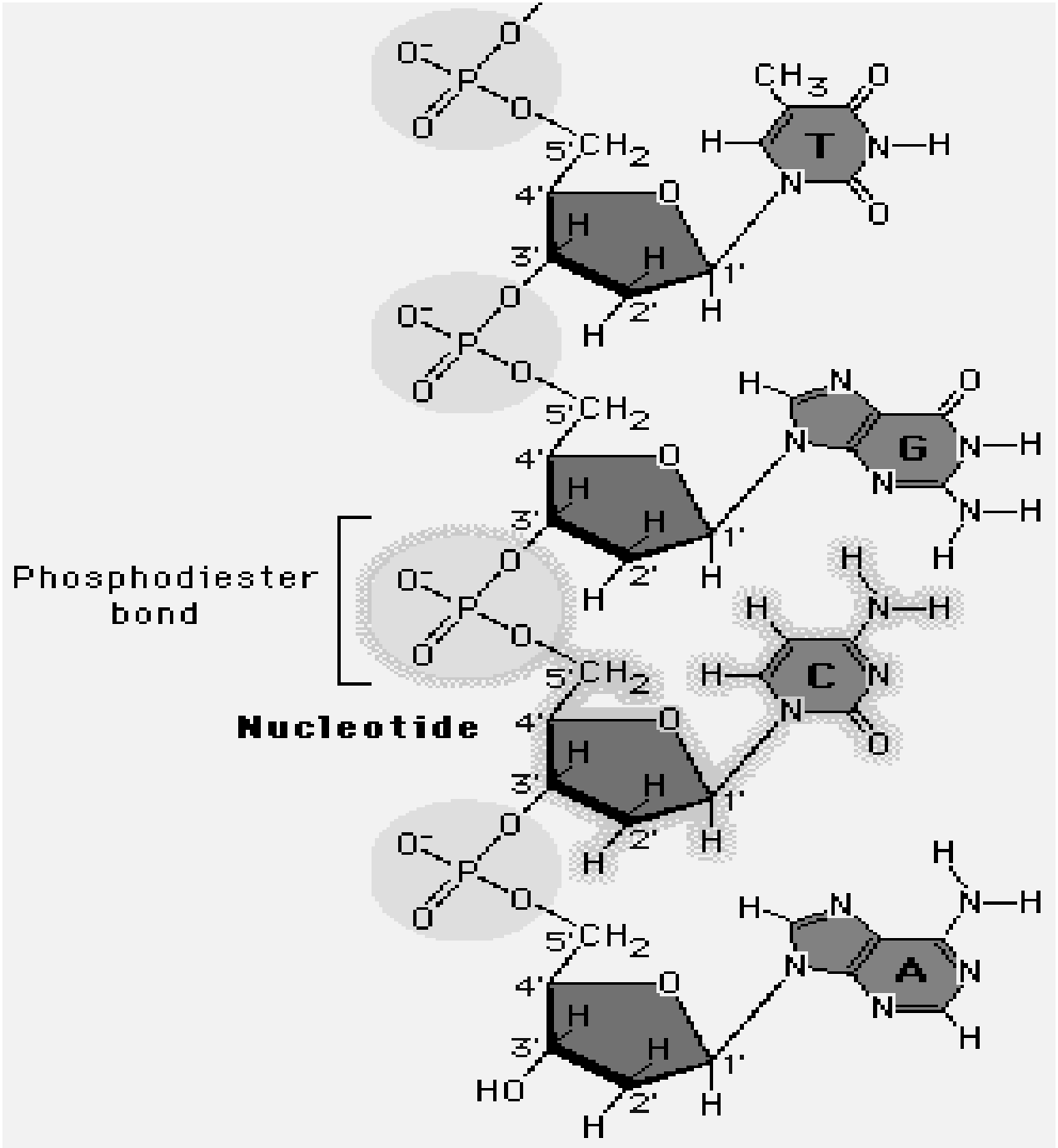
Nucleotide




Cytosine C
Guanine G
Adenine A
Uracil U
replaces Thymine in RNA
Nitrogenous Bases
RNA
Ribonucleic acid
Nitrogenous Bases
Base pair
Sugar phosphate backbone
DNA
Deoxyribonucleic acid
C Cytosine
G Guanine
A Adenine
T Thymine
Nitrogenous Bases

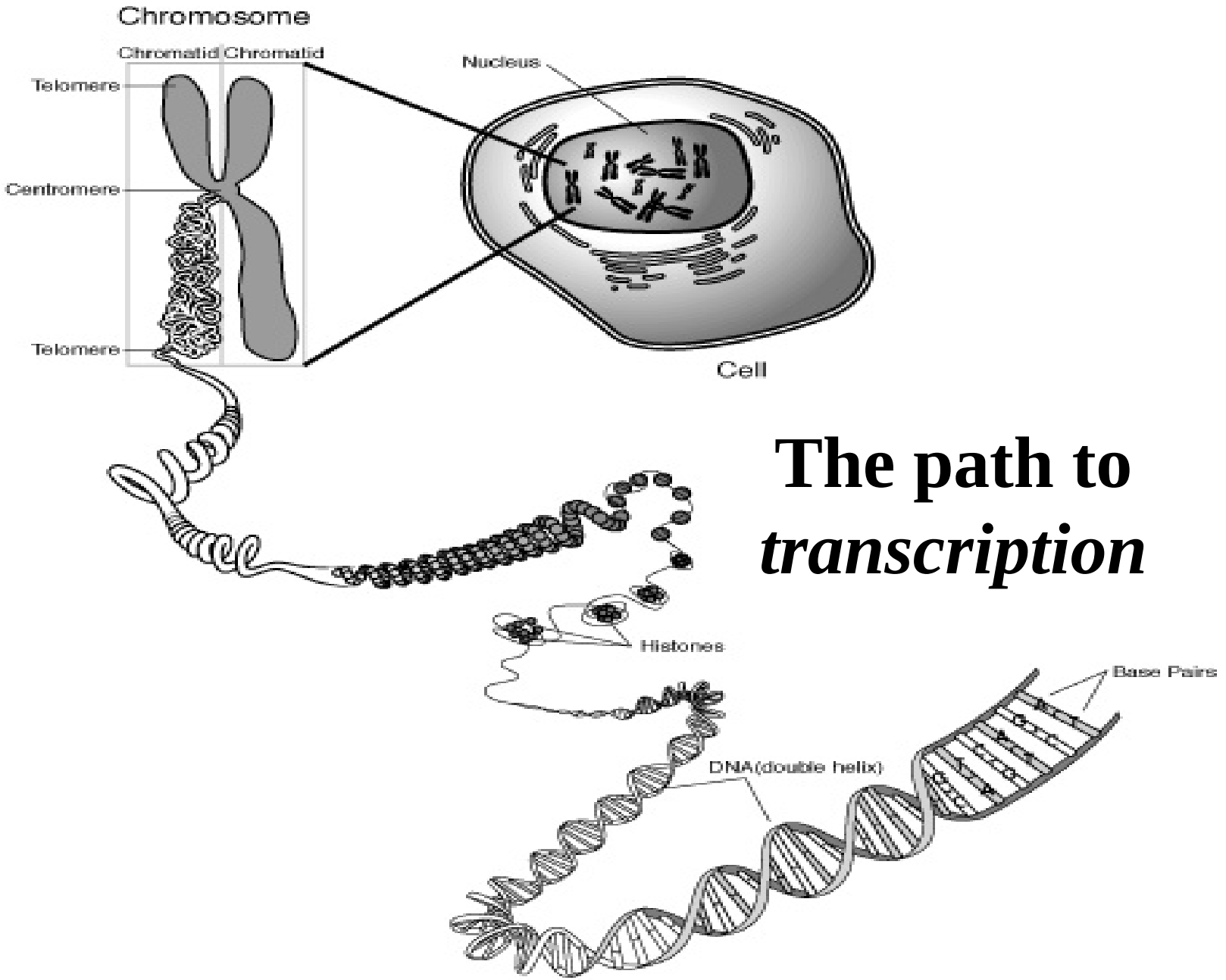
Base Pairing




Phosphodiester bond
Nucleotide
T
G
C
A

Transcription

- RNA polymerase binds to the promoter region of a gene
  - Only one of the DNA strands is transcribed
  - The strand varies from gene to gene
- A complementary strand of RNA, called messenger RNA (mRNA), is produced from the DNA template.
  - RNA polymerase builds the RNA by linking nucleotides in the **5’ ® 3’** direction

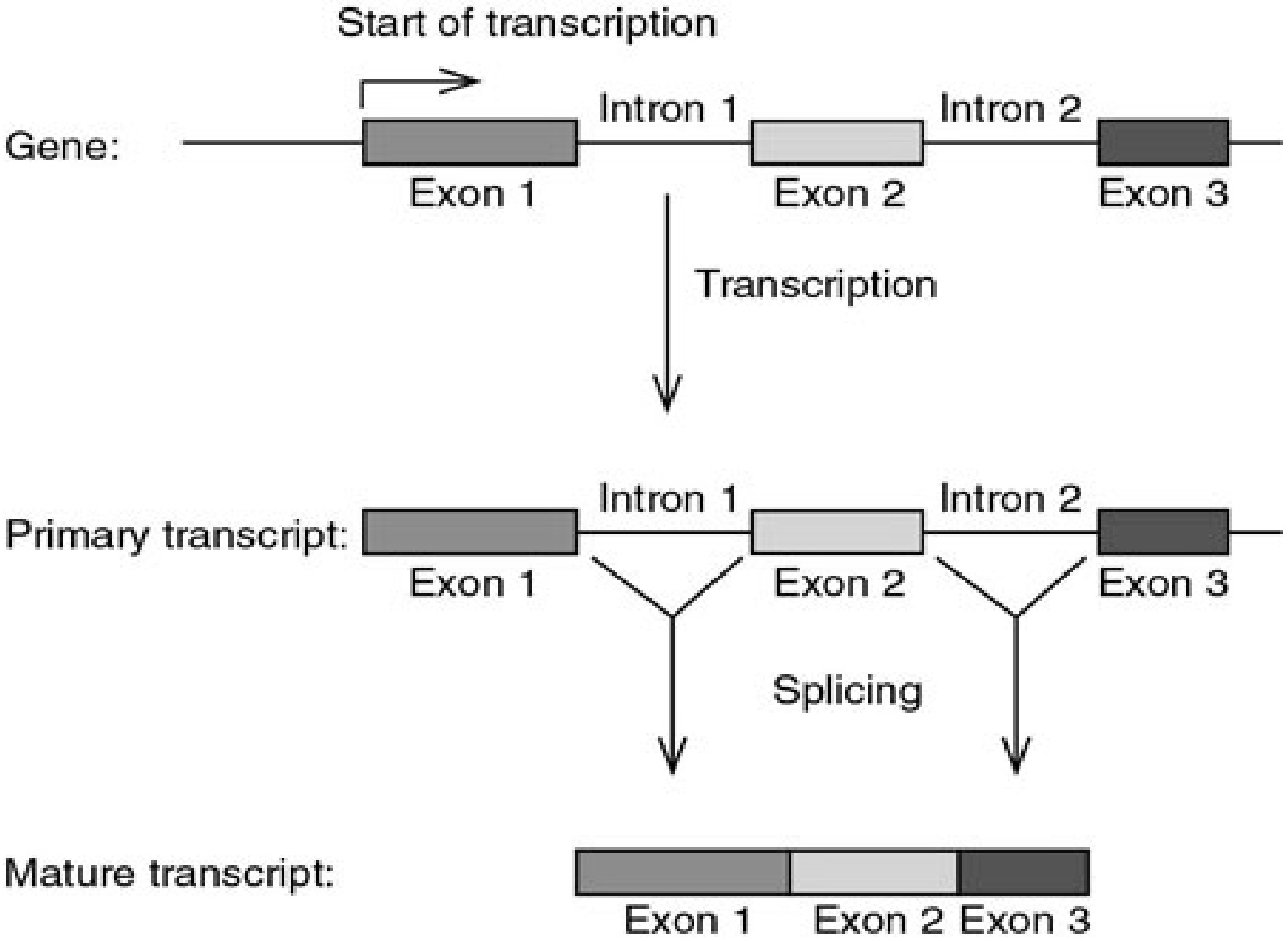


The path to *transcription*

RNA Processing

- In eukaryotes, **RNA is modified** after it is transcribed
  - The ends are modified
  - Intervening sequences are removed
- The modified mRNA moves out of the nucleus and into the cytoplasm

Introns


Start of transcription
Gene:
Intron 1
Intron 2
Exon 1
Exon 2
Exon 3
Transcription
Primary transcript:
Intron 1
Intron 2
Exon 1
Exon 2
Exon 3
Splicing
Mature transcript:
Exon 1
Exon 2
Exon 3

Proteins

- Proteins are used for a variety of functions in the cell:
  - Structural support
  - Metabolism
  - Motion
  - Defense
  - DNA replication, RNA synthesis, etc.
  - And many more functions

Overview of Protein Synthesis

- The information in DNA is used to make proteins
- In order to get from DNA to protein, an intermediary, mRNA, is necessary
- The information in the mRNA is translated into the amino acids chains that make up the protein by molecules of tRNA

Protein Synthesis

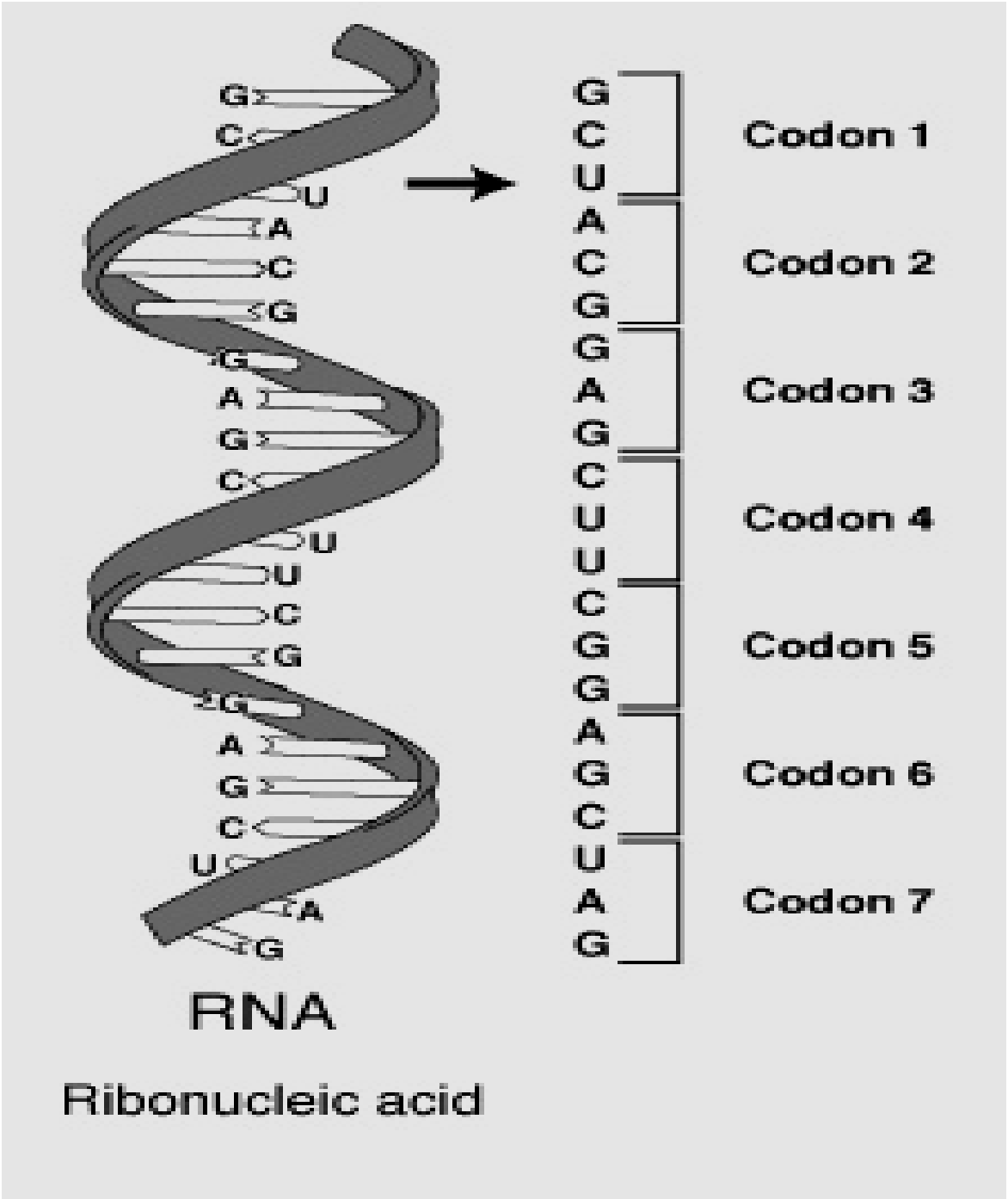
- Every cell in your body has the **same DNA**
- The DNA in the cell has the instructions for making the proteins that the cell needs
  - Different cells need different proteins, and not all proteins are needed all the time
  - Not every protein is made in every cell
- **Genes are regulated so that the necessary proteins are made**

The *Genetic Code*

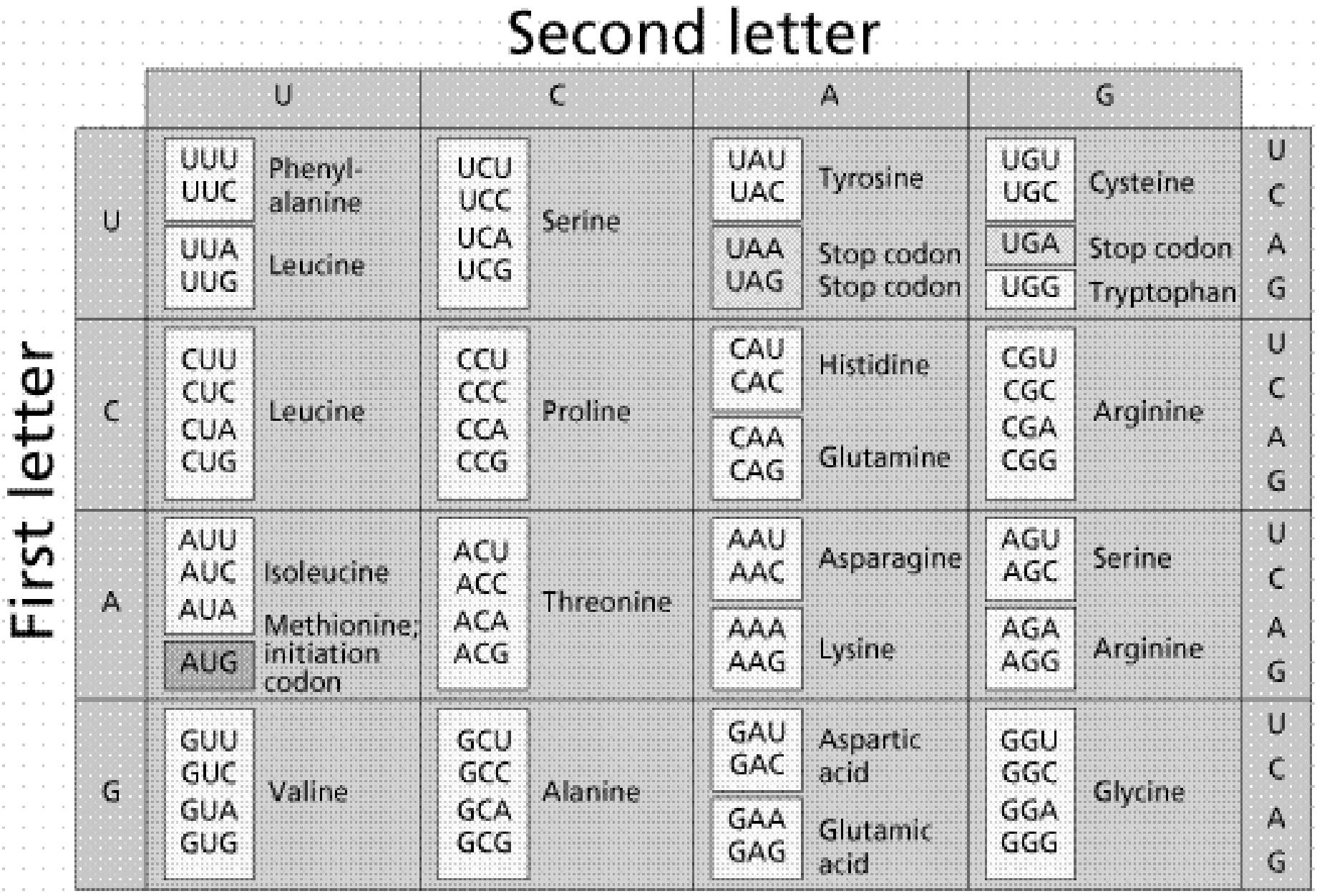
- Information based on a triplet code
  - Codons are the basic unit
    - Groups of 3-nucleotides
  - 64 possible ***codons***
    - 61 specify one of the 20 ***amino acids***
    - three are stop ***codons***
- The ordering and grouping of the nucleotides is called the reading frame

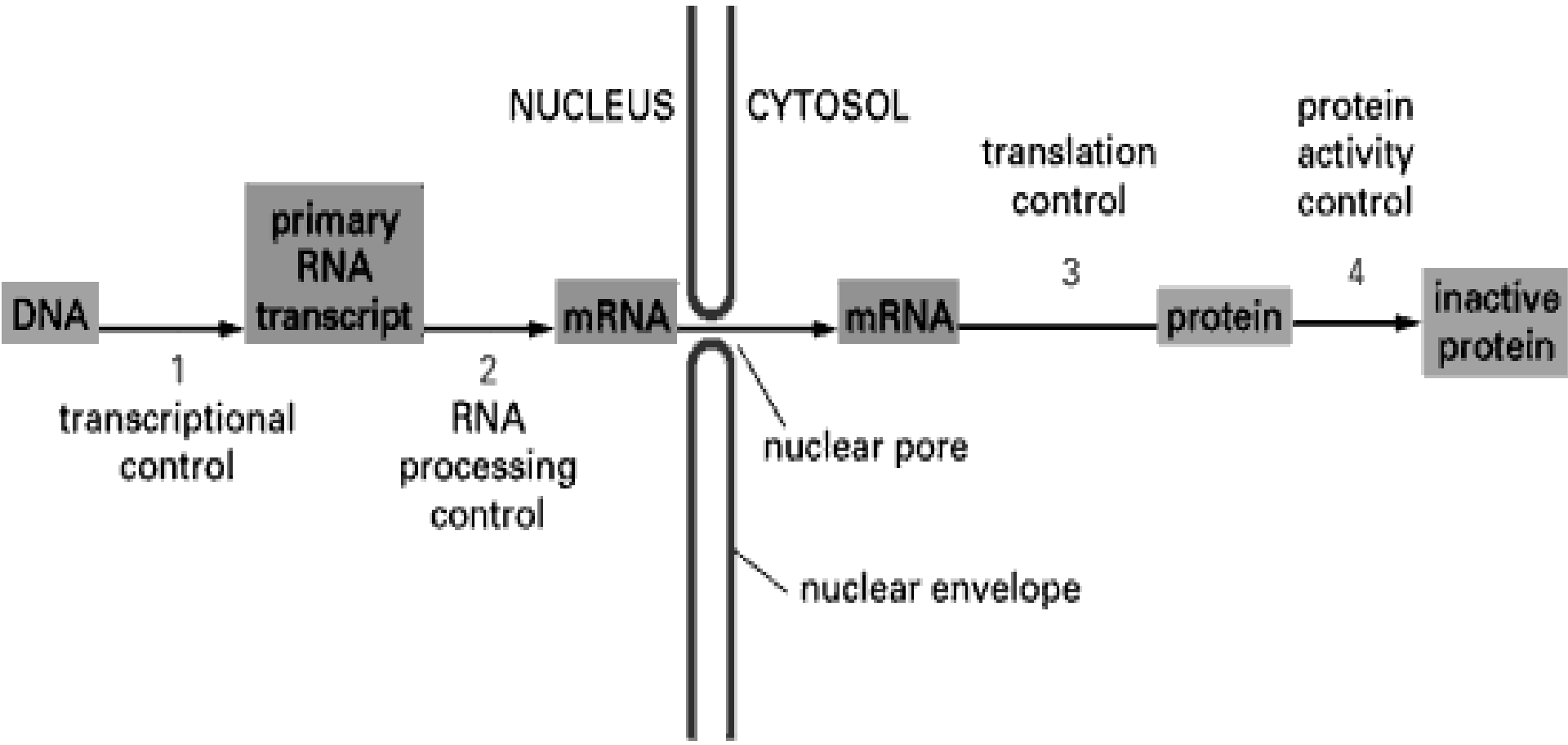
Codons

…letters in the sentence



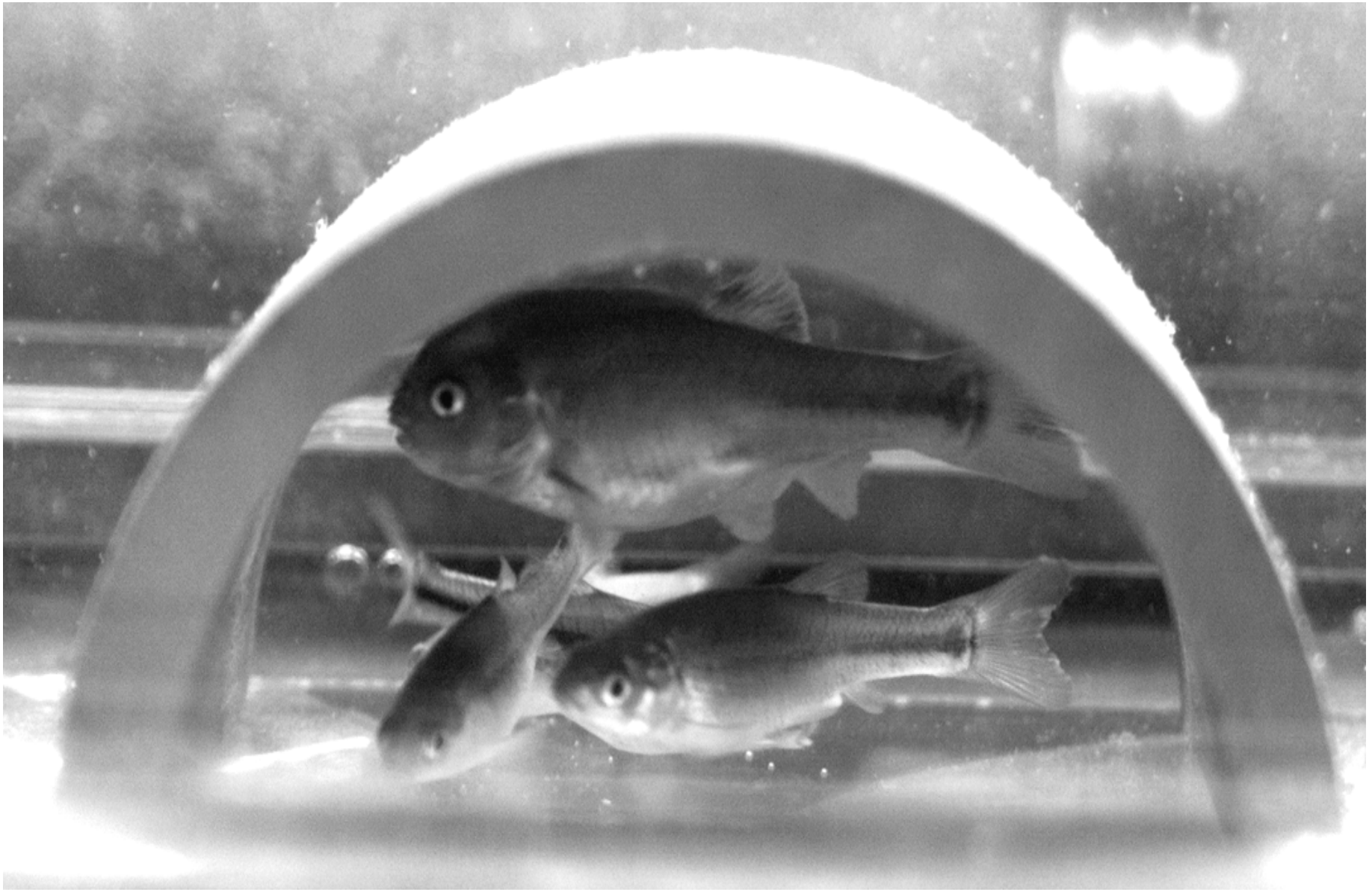
Codon Table

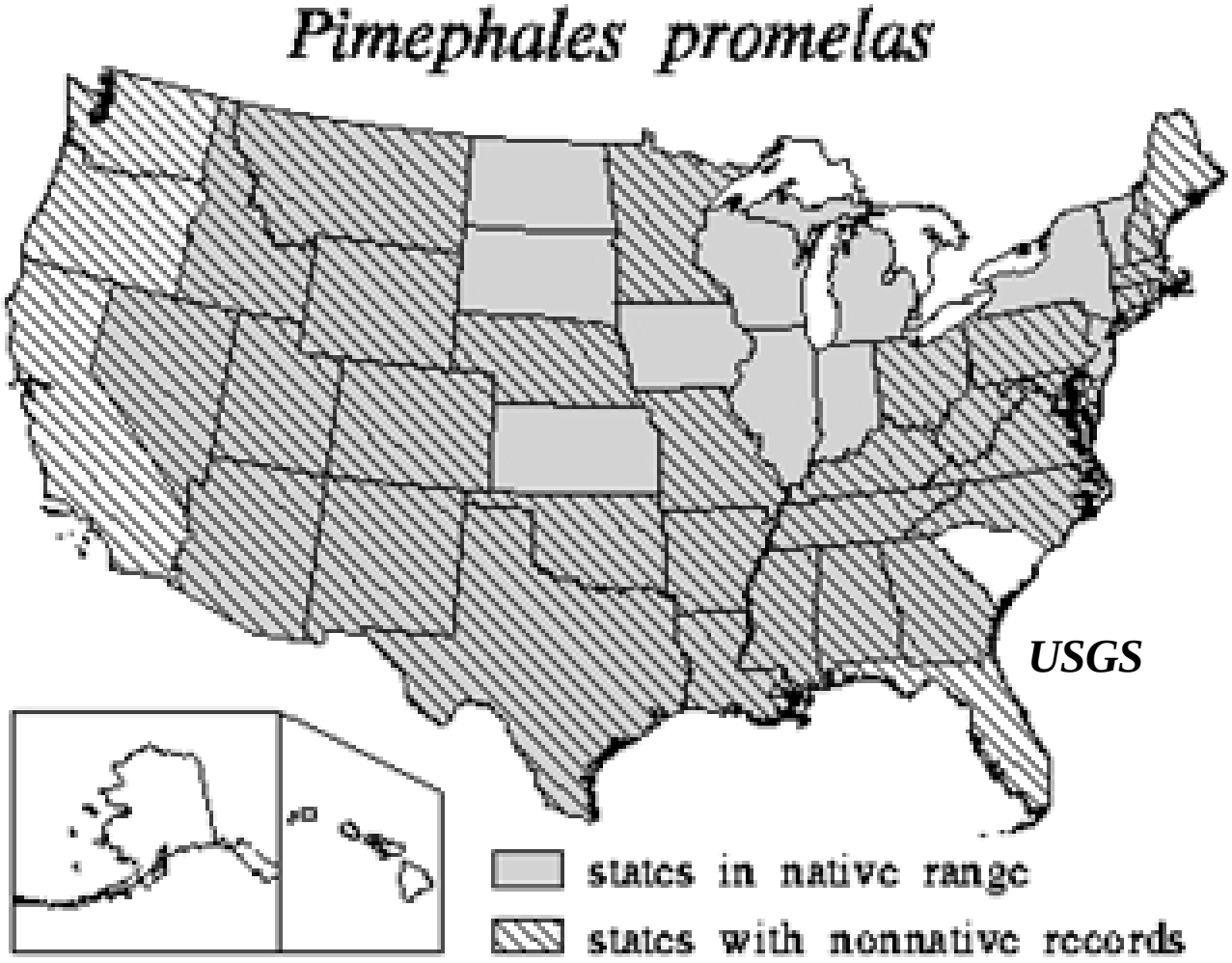

Second letter
First letter
U
C
A
G
UUU UUC Phenyl-alanine
UUA UUG Leucine
UCU UCC UCA UCG Serine
UAU UAC Tyrosine
UAA Stop codon
UAG Stop codon
UGU UGC Cysteine
UGA Stop codon
UGG Tryptophan
CUU CUC CUA CUG Leucine
CCU CCC CCA CCG Proline
CAU CAC Histidine
CAA CAG Glutamine
CGU CGC CGA CGG Arginine
AUU AUC AUA Isoleucine
AUG Methionine; initiation codon
ACU ACC ACA ACG Threonine
AAU AAC Asparagine
AAA AAG Lysine
AGU AGC Serine
AGA AGG Arginine
GUU GUC GUA GUG Valine
GCU GCC GCA GCG Alanine
GAU GAC Aspartic acid
GAA GAG Glutamic acid
GGU GGC GGA GGG Glycine


NUCLEUS
CYTOSOL
translation control
protein activity control
primary RNA transcript
DNA
mRNA
mRNA
protein
inactive protein
3
4
1
transcriptional control
2
RNA processing control
nuclear pore
nuclear envelope

Pimephales promelas

Fathead minnow




Pimephales promelas
USGS
states in native range
states with nonnative records

The Critter -

Sexually ***dimorphic***

Reproductive maturity in
4-5 months; therefore,
rapid generational time…

30 years of toxicological data…

The Gene, *Vitellogenin* -

Exquisitely ***responsive*** to estrogenic compounds and estrogen mimics…

Vitellogenin is normally ***quiescent*** in male fish…

Estrogenic induction detectable within ***two hours***…

A molecular biological approach to aquatic indicators can…

- Provide real time biological indicators of environmental chemical exposure in aquatic systems.
- Allow for detection of the first cellular event in response to environmental impact…far in advance of those effects observed at higher levels of biological organization.
- Offer the most sensitive biological link between exposure events and critical programs in reproduction and development.

A molecular biological approach to aquatic indicators is…

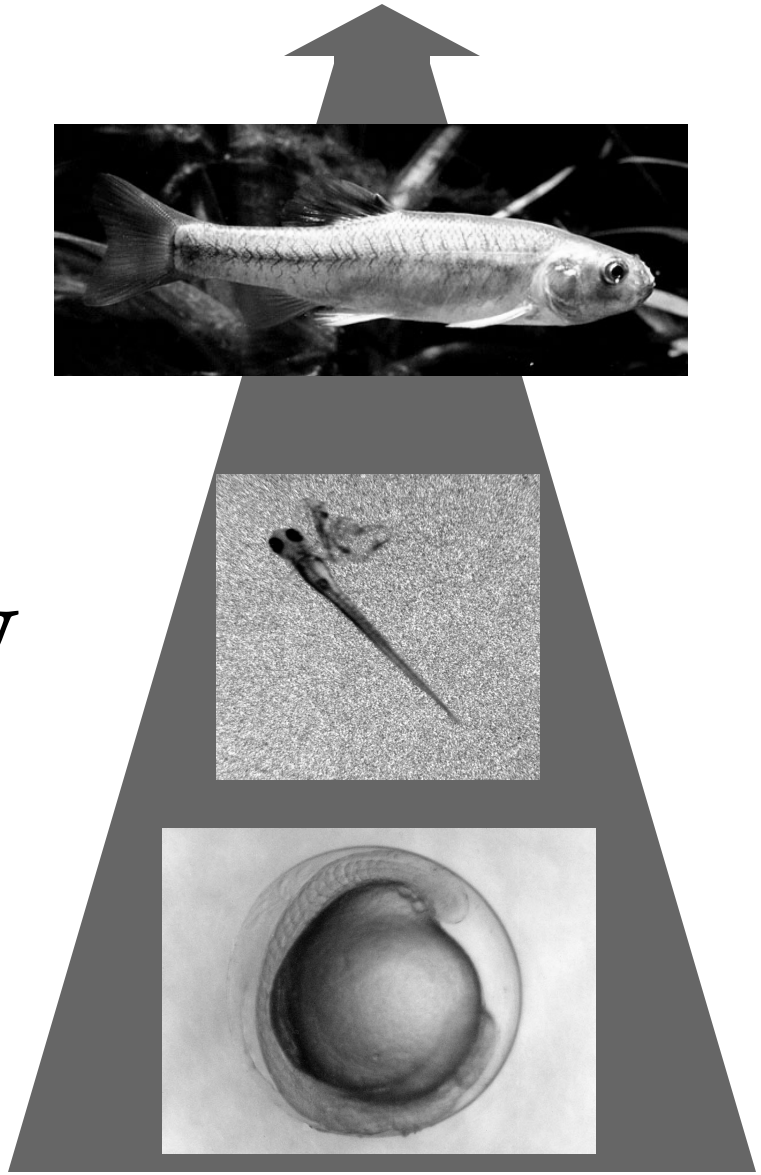
- Able to detect exposure at the onset of the critical biochemical pathway – well in advance of confounding cellular control mechanisms.
- Amenable to technology transfer and standardization across laboratories.
- Rapid and quantifiable…changes in gene expression, following exposure, readily detectable in a matter of hours.

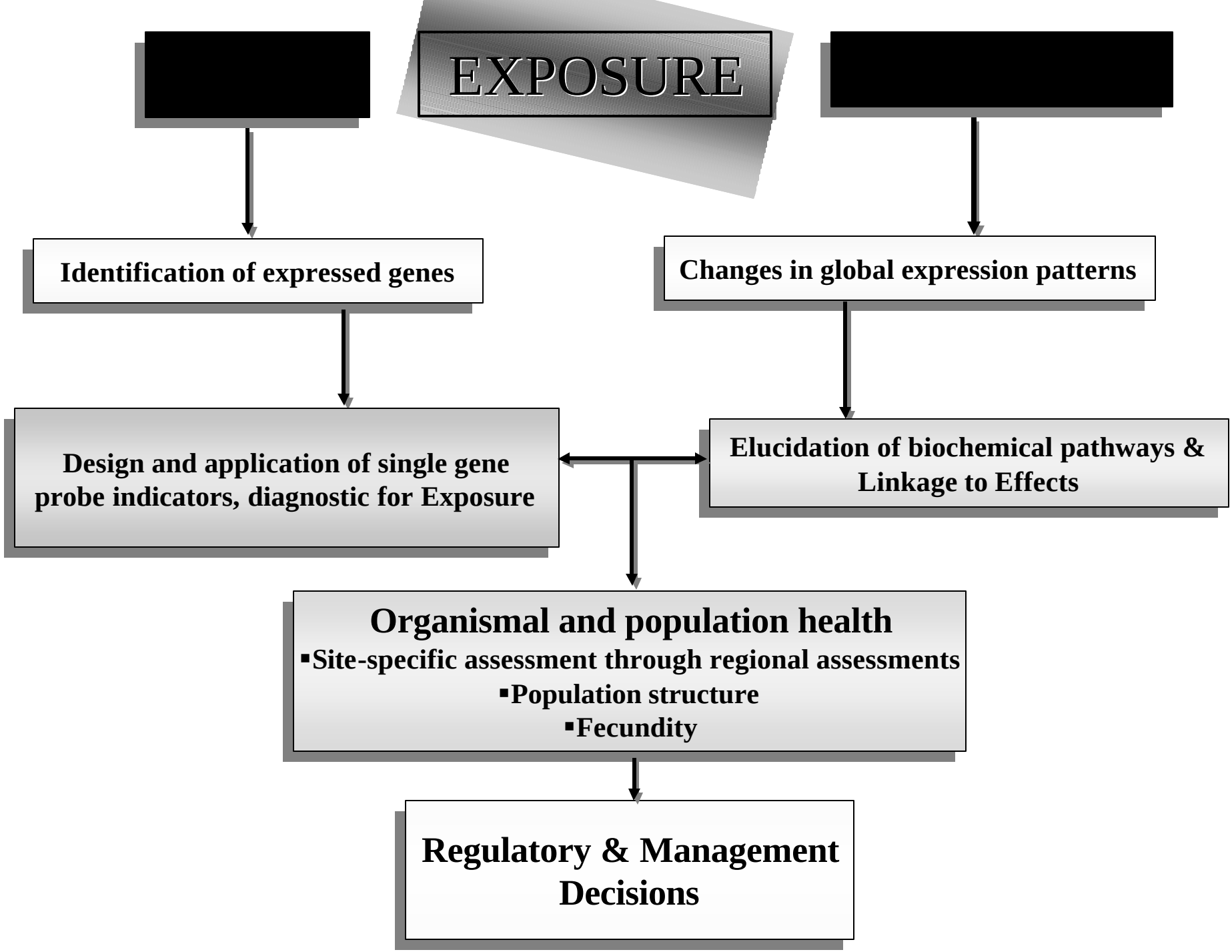
How critical is *life stage*?

Adults

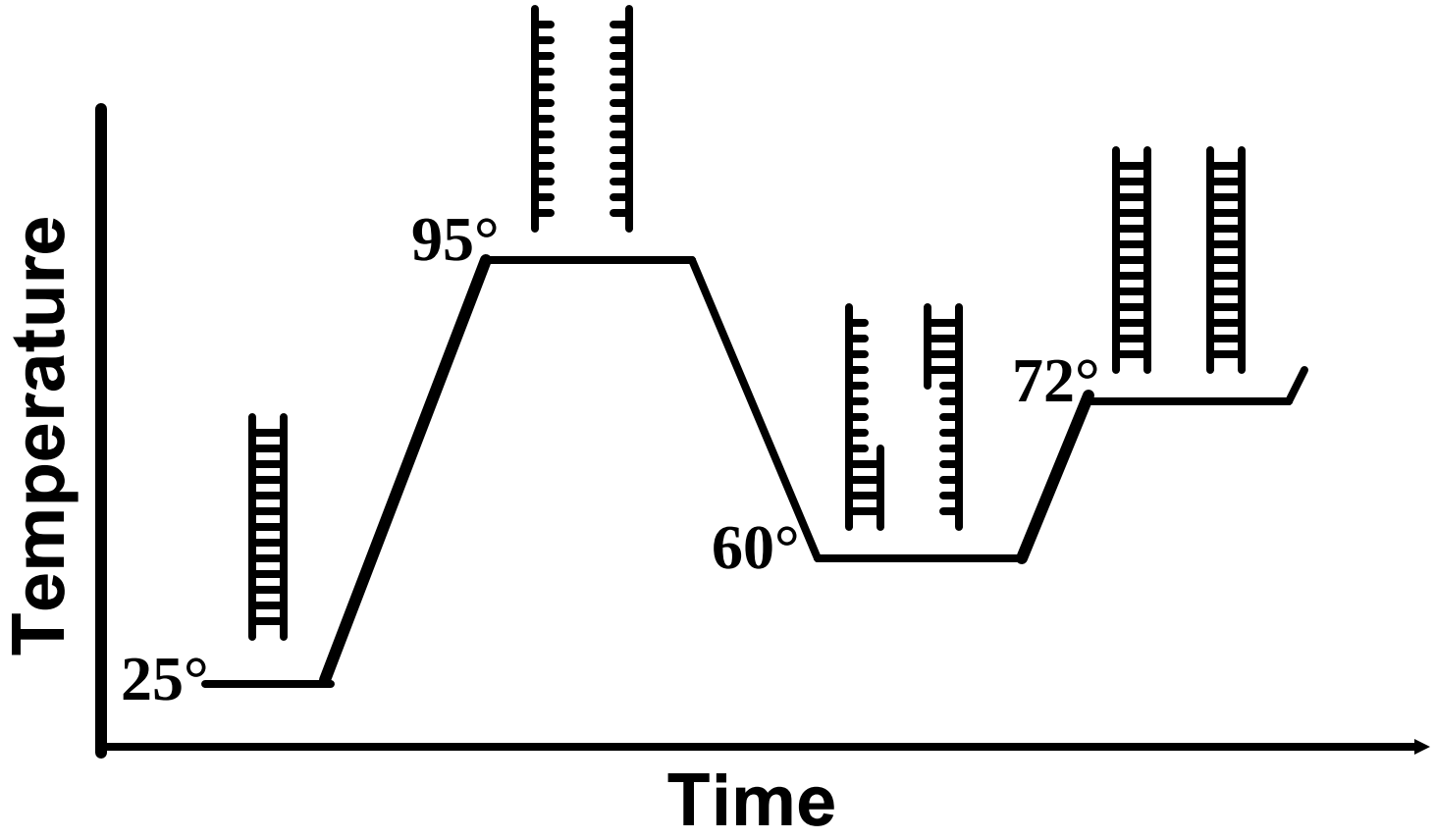
Juvenile & fry

Embryos




EXPOSURE
Identification of expressed genes
Changes in global expression patterns
Design and application of single gene probe indicators, diagnostic for Exposure
Elucidation of biochemical pathways & Linkage to Effects
Organismal and population health
▪Site-specific assessment through regional assessments
▪Population structure
▪Fecundity
Regulatory & Management Decisions

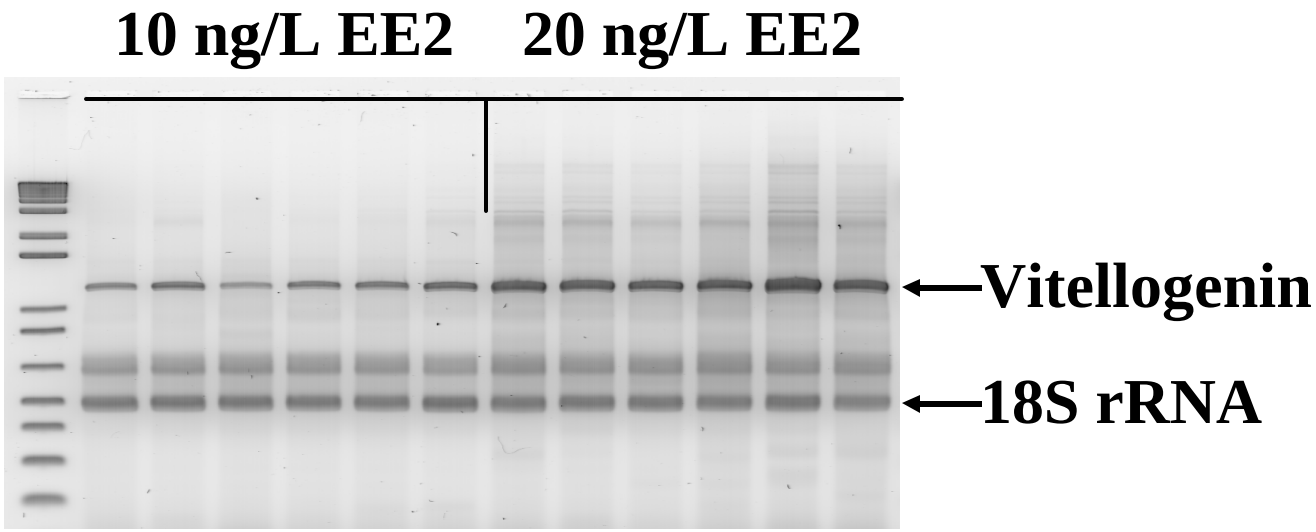
PCR: One cycle of amplification



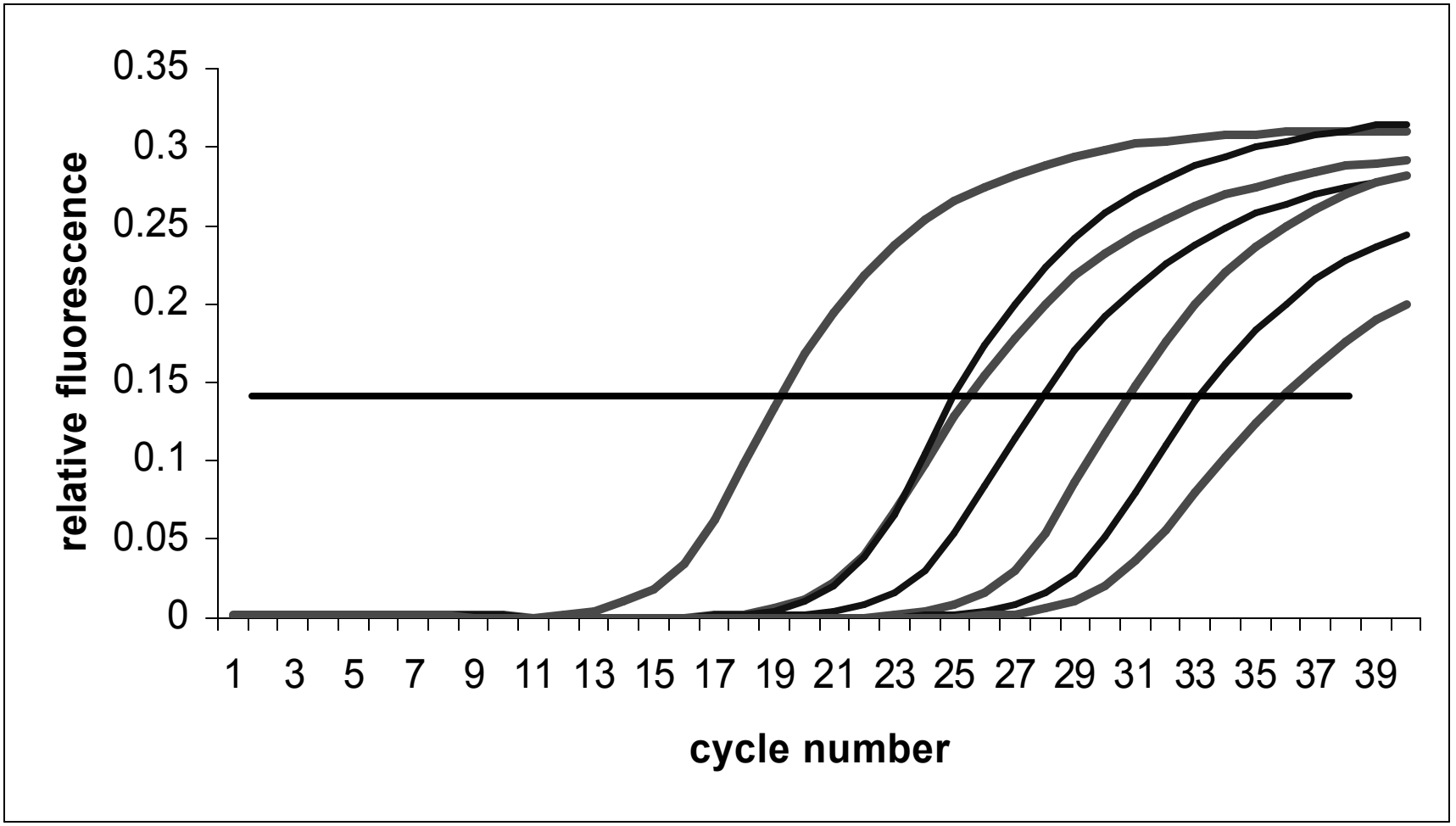
Vitellogenin gene expression

Pimephales promelas embryo larvae

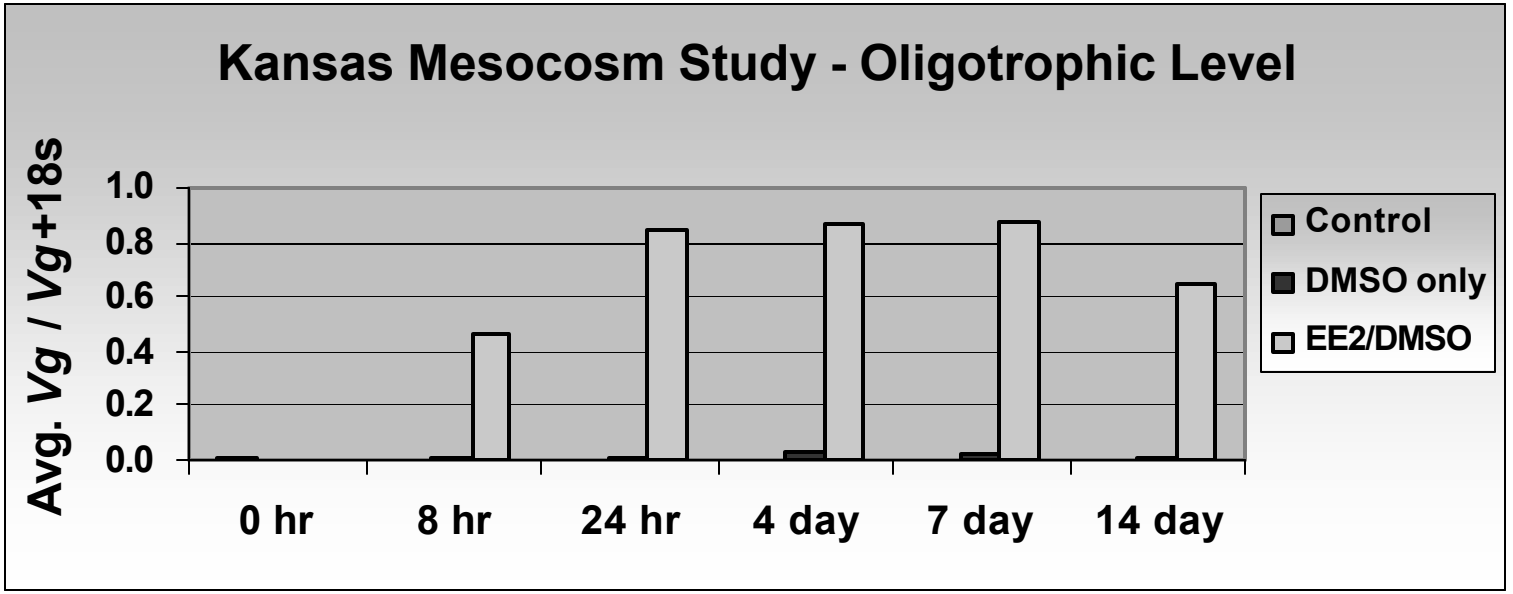
24 hour exposure

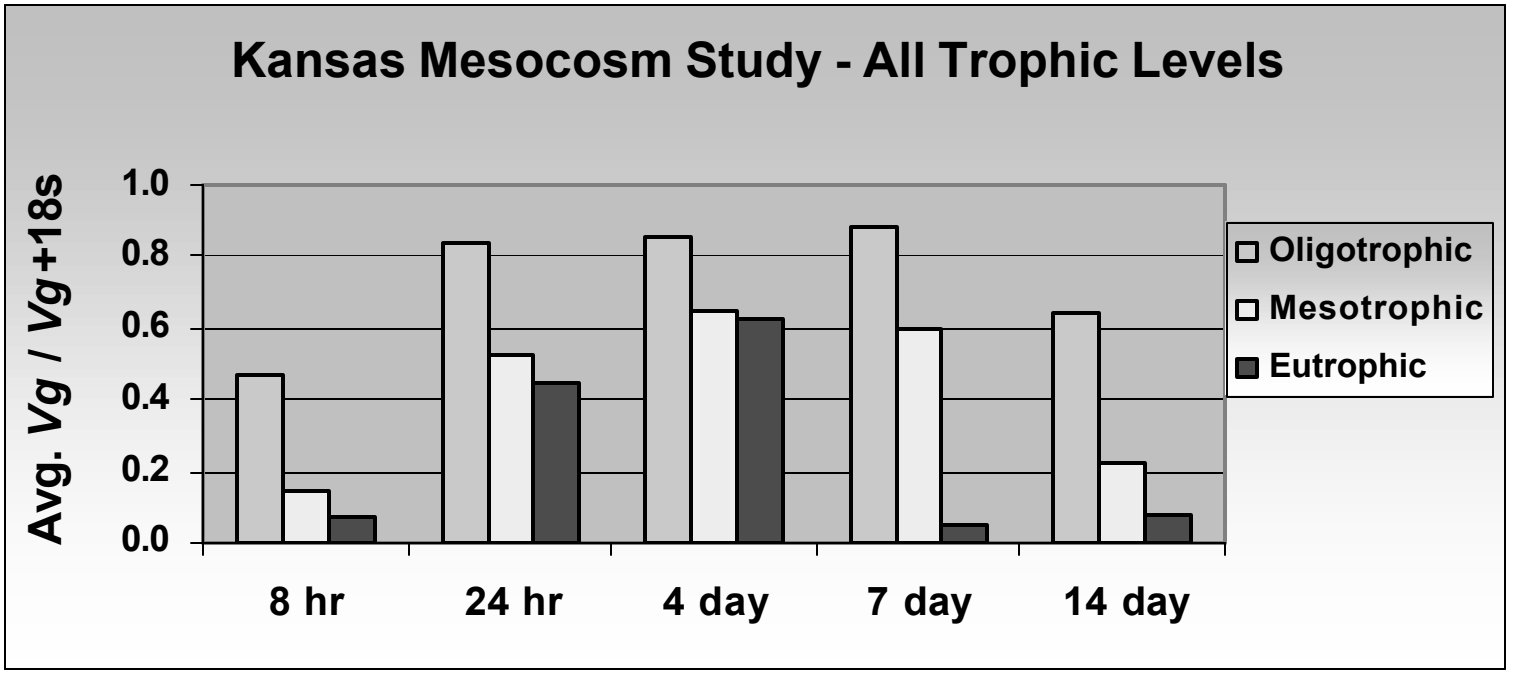


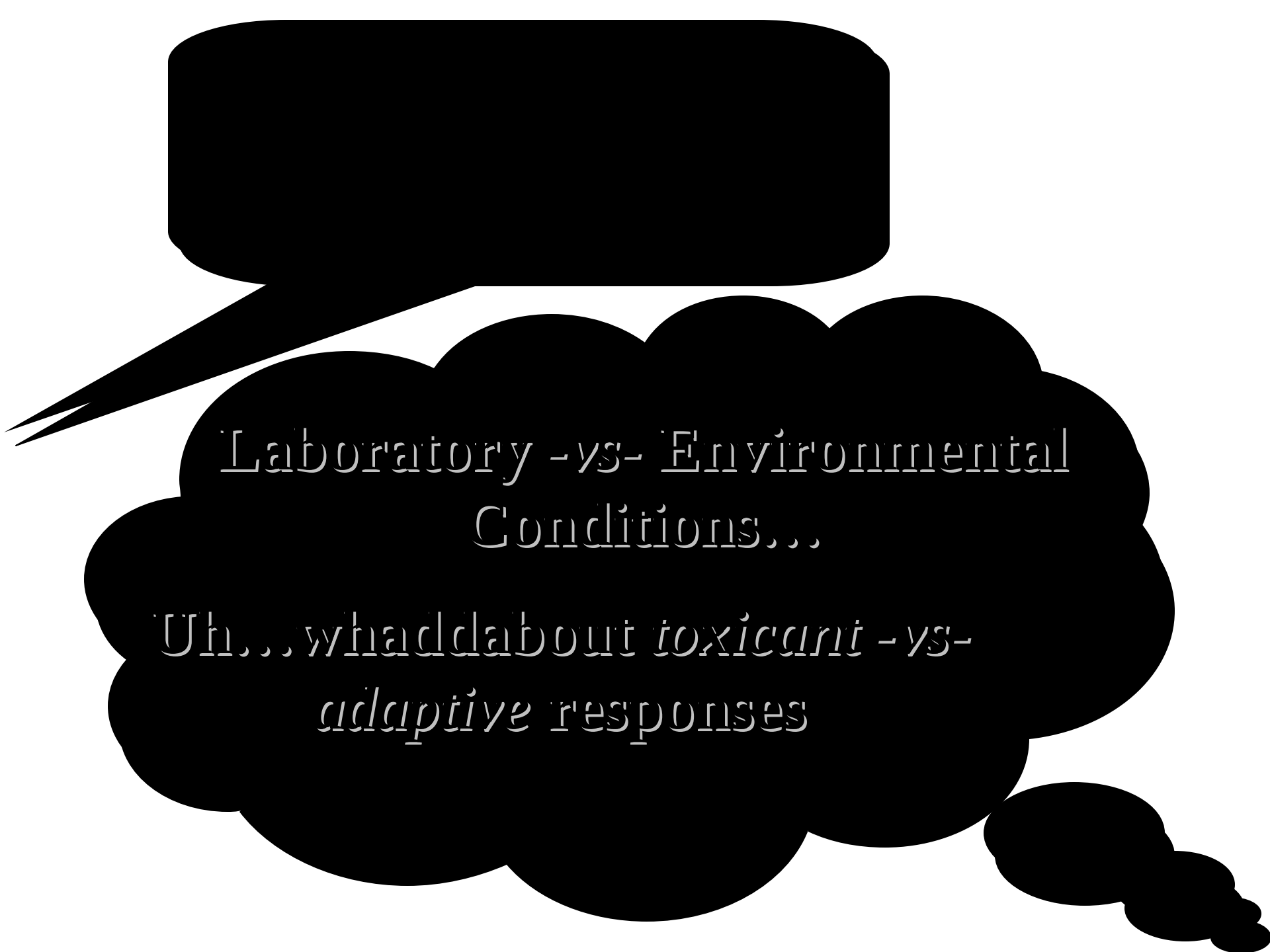
"*Real-Time*" visualization of amplified products



Part of the *vitellogenin* story…

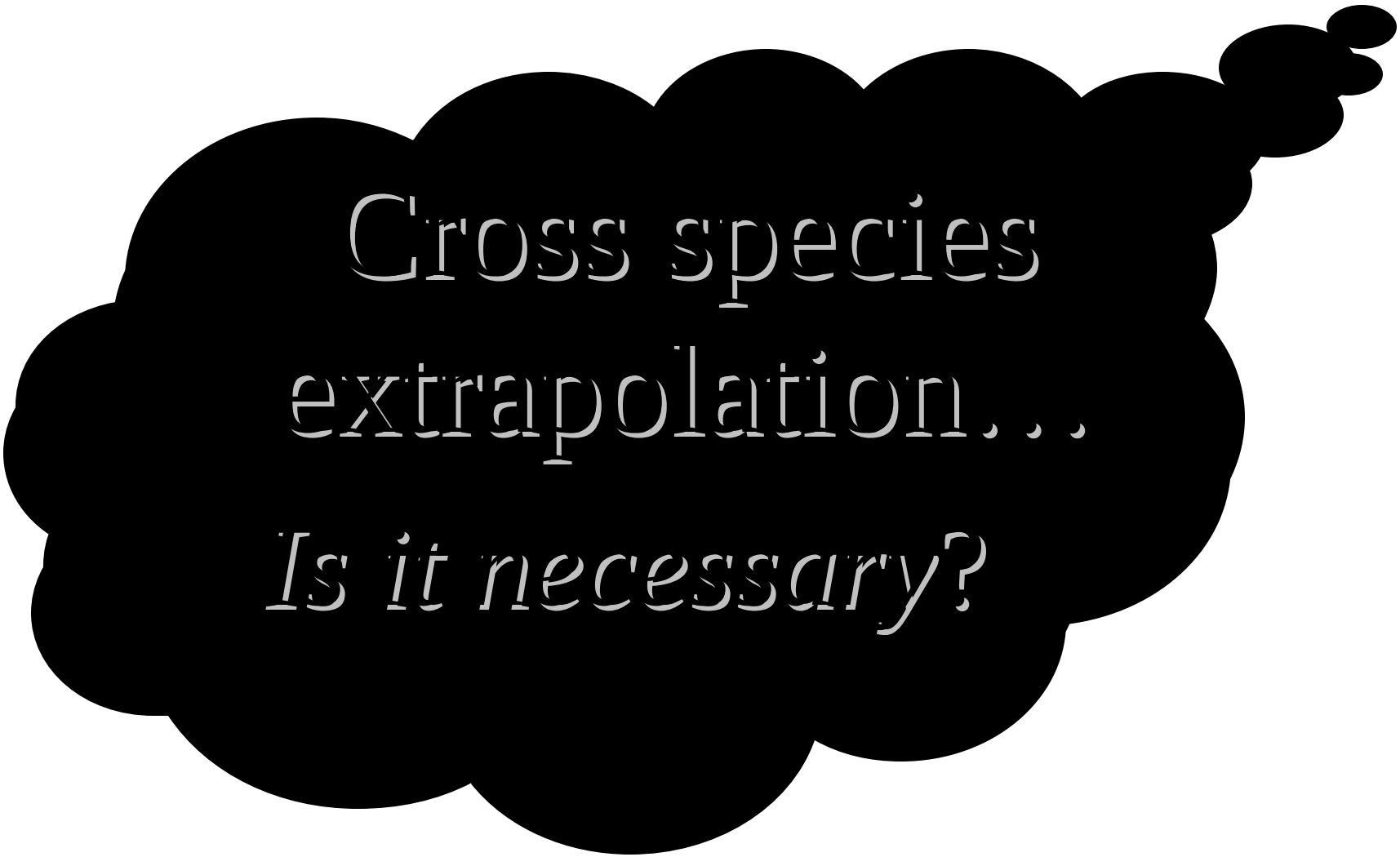





Laboratory -vs- Environmental Conditions...
Uh...whaddabout *toxicant* -vs- *adaptive* responses


Exposures…
Environmentally relevant concentrations?
Duration?
Chronic -*vs*- Acute


Mixtures…
Inhibition?
Additivity?
Synergism?


Cross species extrapolation…
Is it necessary?

The need for *scientific integration*

Observations stemming from the global molecular approach are not entirely sufficient, and must be considered in context of other disciplines such as ***toxicology, ecology, population biology, physiology and behavior***.

The culpable individuals

Ann Miracle
Denise Gordon
Robert Flick
Jim Lazorchak
T.V. Reddy
Gregory Toth